

Table S1. Primers used in this study.

Locus	Primer name	Sequence (5' to 3')	Location (<i>D. melanogaster</i>)	Notes
<i>tpi</i>	Tpi-F	CAACTGGAAGATGAAAYGGIGACC	3 rd chromosome	From Shoemaker et al. 2004
	Tpi-R	TTCTTGGCATAGGCGCACATYTG		
<i>skpA</i>	skpA-F	AAVATGCCBARYATYAARYTGCARTC	X chromosome	From Dyer et al. 2005
	skpA-R	CTTCTCCTCRCACCAAYTCRTTC		
<i>RpL36</i>	Rpl-F	CMRVGSCCACAAGACCWCSAARRTC	X chromosome	From Dyer et al. 2005
	Rpl-R	CRTGRGTCTGRGCCTTCC		
<i>pgd</i>	Pgd-F	ATYGATGGYGGCAACTC	X chromosome	From Dyer et al. 2005
	Pgd-R	CNCGCATWAGCATRAAKCCYTG		
<i>mof</i>	Mof-F	CAGAAGCGRCGCTACGA	X chromosome	From Dyer et al. 2005
	Mof-R	TAKGCCCAATAGCTGCGATA		
<i>bt</i>	Bt-F4	CYGAYTGGGAYAARGATCACATYG	Dot chromosome	
	Bt-R3	AYTCRTATTTRACRCCYTCAATCAAGTC		
<i>gw</i>	Gw-F	GCATGATTKRTCCWCGGG	Dot chromosome	
	Gw-R	GTTGGATTTTGKCKCKCATTAA		
<i>plexA</i>	plexA-F8	TATGTACTIONCGAYCCAACWGTTG	Dot chromosome	
	plexA-R8	CACAAGCARRGCAACAAAGATC		
	plexA-F7	CMCAATTTACCAGYATAACAAGC		
	plexA-R1	GCARTGTGACCATTATTAATGAGG		
<i>eIF4G</i>	eIF4G-F8	GGTGCGKGGWATTYTAAYAAAG	Dot chromosome	
	eIF4G-R8	AWATATTGYGCAGATARCTGCTC		
	eIF4G-F9	CCMGARAGRTTYGAYACRCTGG		
	eIF4G-R4	GCCTCCTTTTCRATTTGTCC		
<i>kl-2</i>	K12-F20	CWGAAGTTAARTCATATGGRCG	Y chromosome	Exon 7 in <i>D. melanogaster</i>
	K12-R778	GACCTTGAATATTCATTCTCG		
<i>kl-3</i>	K13-F11	GGWAGCGTTGARCTTTGG	Y chromosome	Exon 1 in <i>D. melanogaster</i>
	K13-R11	CRTGSCGCACCAGTGATG		
<i>kl-5</i>	K15-F8	KTTYGARYTRCAAGGKCCAGATCC	Y chromosome	Exon 12 in <i>D. melanogaster</i>
	K15-R6	GCSGGCCAYTCRAATATCCAMAC		
	K15-F3	CAGTGGKAARGTWGARGTKTGG		
	K15-R5	CRGGSACMACCATTGCACARG		
	K15-F30-Y	GCCCTGGAAGAAAATCGAC		
	K15-R30-Y	CATAGCCAGGGTTCATAGTTATAAAA		
	K15-F30-A	GCCCTGGAAGAAAATCGAT		Exon 12 in <i>D. melanogaster</i>

K15-R30-A	CATAGCCAGGGTTCATAGTTATAAAT	Exon 12 in <i>D. melanogaster</i>
K15-F44	GCWGARGCYAGAGACATAACWAARTG	Exon 3 in <i>D. melanogaster</i>
K15-R46	CAKGTCCARTCCTGYGAGTTAAATG	Exon 5 in <i>D. melanogaster</i>
K15-F41	ATGAARACRGARTCWCAAGARATG	Exon 2 in <i>D. melanogaster</i>
K15-R43	TGTCTCTRGCYTCSGCYAAAGATTC	Exon 3 in <i>D. melanogaster</i>
K15-R47	TRTACTGSATATTKGTCCAAYTCACG	Exon 6 in <i>D. melanogaster</i>

Table S3. *kl-5* intron locations and sizes.

	Intron 2	Intron 3	Intron 4	Intron 5
	In aa 125	After aa 238	In aa 328	In aa378
<i>D. melanogaster</i> (Y)	57	57	56	86
<i>D. hydei</i> (Y)	No amplification (intron 4)	54 (intron 5)	65 (intron 6)	72 (intron 7)
<i>D. pseudoobscura</i> (A)	82 (intron 4)	55 (intron 5)	60 (intron 6)	unknown /not annotated
<i>D. putrida</i> (Y)	No amplification	44	57	No amplification
<i>D. neotestacea</i> (A)	316	44	57	75
<i>D. testacea</i> (A)	323	44	57	75
<i>D. orientacea</i> (A)	137	44	57	75
<i>D. orientacea</i> (Y)	Unknown	44	57	75

Species in the testacea group are shown relative to *D. melanogaster* (Genbank accession CG40444), *D. hydei* (Genbank accession AF031494; Kurek et al. 2000), and *D. pseudoobscura* (Genbank accession GA26646). Intron number and locations, given by amino acid (aa) of coding sequence, are annotated in *D. melanogaster*. Introns numbers for *D. hydei* and *D. pseudoobscura* are as they are annotated, and shown here as they align to *D. melanogaster*. All sizes shown in base pairs (bp). The A (autosomal) or Y (Y-chromosome) indicates genomic position. In *D. orientacea* the autosomal and Y-linked *kl-5* copies are of identical size for introns 3, 4, and 5, as indicated by male-specific double peaks on the chromatograms. It is unknown whether intron 2 occurs on the Y-linked copy of *kl-5* in *D. orientacea* because no male-specific double peaks were identified on the chromatograms. No amplification for intron 1 or introns 6-12 was attempted.

Table S4. *kl-5* divergence among testacea group species.

	<i>D. putrida</i>	<i>D. neotestacea</i>	<i>D. testacea</i>	<i>D. orientacea</i> (A)	<i>D. orientacea</i> (Y)
<i>D. putrida</i> (Y)		0.053	0.055	0.062	0.067
<i>D. neotestacea</i> (A)	0.008, 0.140		0.070	0.113	0.127
<i>D. testacea</i> (A)	0.009, 0.155	0.003, 0.040		0.075	0.087
<i>D. orientacea</i> (A)	0.011, 0.162	0.007, 0.052	0.005, 0.067		0.065
<i>D. orientacea</i> (Y)	0.012, 0.165	0.007, 0.055	0.006, 0.069	0.002, 0.025	

1800 bp of coding sequence included. K_A and K_S are in the lower diagonal, K_A/K_S in the upper diagonal. The A (autosomal) and Y (Y-chromosome) indicate genomic position.

Table S5. Polymorphism and divergence of loci in the testacea group species.

species	gene	chr	N	All sites					Silent sites					π_A/π_S	K_A	K_S	K_A/K_S
				L	S	π	θ	D	L	S	π	θ	D				
<i>putrida</i>	<i>bt</i>	dot	10	793	2	0.09	0.09	0.12	188.0	2	0.39	0.38	0.12	0	-	-	-
	<i>eIF4G</i>	dot	11	753	1	0.07	0.05	1.19	168.7	1	0.30	0.20	1.19	0			
	<i>gw</i>	dot	1	428	-	-	-	-	104.5	-	-	-	-	-	-	-	-
	<i>plexA</i>	dot	8	1097	2	0.06	0.07	-0.45	250	2	0.27	0.31	-0.45	0	-	-	
	<i>kl-2</i>	Y	8	725	0	0	0	-	168.0	0	0	0	-	0	-	-	-
	<i>kl-3</i>	Y	6	840	0	0	0	-	170.8	0	0	0	-	0	-	-	-
	<i>mof</i>	X	10	615	30	1.56	1.72	-0.45	138.7	30	6.923	7.648	-0.45	0	-	-	-
	<i>pgd</i>	X	10	663	49	1.97	2.61	-1.12	155.0	43	7.47	9.81	-1.07	0.038	-	-	-
	<i>RpL36</i>	X	10	313	7	0.76	0.79	-0.17	119.6	7	1.99	2.07	-0.43	0	-	-	-
	<i>skpA</i>	X	10	432	2	0.17	0.16	0.12	89.2	2	0.82	0.79	0.12	0	-	-	-
<i>tpi</i>	A	16	362	11	0.72	0.92	-0.82	88.2	9	2.66	3.08	-0.50	0.034	-	-	-	
<i>orientacea</i>	<i>bt</i>	dot	6	793	1	0.04	0.06	-0.93	188.2	0	0	0	-	-	0.017	0.103	0.155
	<i>eIF4G</i>	dot	4	753	0	0	0	-	169.5	0	0	0	-	0	0.009	0.168	0.051
	<i>gw</i>	dot	7	428	2	0.13	0.19	-1.24	103.7	1	0.28	0.39	-1.01	0.322	0.010	0.175	0.049
	<i>plexA</i>	dot	5	1097	2	0.09	0.09	0.24	251.2	2	0.40	0.38	0.24	0	0.004	0.127	0.028
	<i>kl-2</i>	Y	8	725	1	0.03	0.05	-1.05	168.8	1	0.15	0.23	-1.05	0	0.022	0.161	0.121
	<i>kl-3</i>	Y	7	871	1	0.03	0.05	-1.01	270.5	0	0	0	-1.01	0	0.014	0.175	0.070
	<i>mof</i>	X	10	615	18	0.83	1.04	-0.93	139.4	17	3.506	4.31	-0.87	0.012	0.012	0.129	0.084
	<i>pgd</i>	X	10	663	18	0.75	0.96	-0.55	153.7	16	2.75	NA	-0.46	0.053	0.010	0.155	0.056
	<i>RpL36</i>	X	10	313	2	0.13	0.23	-1.40	119.3	1	0.17	0.30	-	-	0.001	0.026	0.020

	<i>skpA</i>	X	10	432	1	0.05	0.08	-1.11	88.8	1	0.23	0.40	-1.11	0	0.003	0.142	0.019
	<i>tpi</i>	A	16	362	16	0.82	1.33	-1.35	86.9	16	0.82	1.33	-1.35	0.014	0.012	0.075	0.149
<i>testacea</i>	<i>bt</i>	dot	10	793	0	0	0	-	189.7	0	0	0	-	0	0.015	0.124	0.111
	<i>eIF4G</i>	dot	14	753	0	0	0	-	169.5	0	0	0	-	0	0.009	0.175	0.049
	<i>gw</i>	dot	12	428	0	0	0	-	104.2	0	0	0	-	0	0.012	0.182	0.060
	<i>plexA</i>	dot	15	1097	4	0.16	0.11	1.21	246.1	4	0.68	0.49	1.21	0	0.007	0.150	0.048
	<i>kl-2</i>	Y	8	726	0	0	0	-	169.8	0	0	0	-	0	0.029	0.178	0.145
	<i>kl-3</i>	Y	7	834	0	0	0	-	261.5	0	0	0	-	0	0.016	0.185	0.075
	<i>mof</i>	X	13	615	13	0.52	0.75	-1.36	139.4	13	2.312	3.297	-1.36	0	0.012	0.129	0.083
	<i>pgd</i>	X	9	663	12	0.60	0.67	-0.51	154.5	10	2.27	2.38	-0.38	0.038	0.009	0.144	0.056
	<i>RpL36</i>	X	10	313	2	0.18	0.23	-0.69	119.3	2	0.47	0.59	-0.69	0	0.000	0.031	0.000
	<i>skpA</i>	X	9	432	3	0.19	0.26	-0.94	88.8	3	0.94	1.24	-0.94	0	0.003	0.143	0.018
	<i>tpi</i>	A	9	362	9	0.62	0.96	-1.72	87.6	7	2.00	3.08	-1.67	0.091	0.009	0.074	0.118
<i>neotestacea</i>	<i>bt</i>	dot	7	793	0	0	0	-	189.8	0	0	0	-	0	0.015	0.103	0.136
	<i>eIF4G</i>	dot	15	753	0	0	0	-	169.5	0	0	0	-	0	0.009	0.152	0.056
	<i>gw</i>	dot	13	428	0	0	0	-	104.5	0	0	0	-	0	0.010	0.163	0.051
	<i>plexA</i>	dot	16	1094	1	0.02	0.03	-0.45	249.5	1	0.09	0.12	-0.45	0	0.007	0.148	0.048
	<i>kl-2</i>	Y	9	725	0	0	0	-	167.7	0	0	0	-	0	0.023	0.149	0.143
	<i>kl-3</i>	Y	8	1444	1	0.02	0.03	-1.05	399.2	1	0.06	0.10	-1.05	0	0.014	0.162	0.077
	<i>mof</i>	X	20	615	5	0.16	0.29	-1.74	138.8	5	0.72	1.273	-1.74	0	0.012	0.135	0.079
	<i>pgd</i>	X	10	663	14	0.61	0.75	-0.84	154.3	12	2.26	2.75	-0.80	0.048	0.010	0.151	0.058
	<i>RpL36</i>	X	11	312	4	0.23	0.44	-1.71	119.7	4	0.61	1.15	-1.60	0	0.000	0.031	0.000
	<i>skpA</i>	X	10	432	6	0.35	0.49	-1.19	89.2	6	1.70	2.39	-1.19	0	0.003	0.145	0.018
	<i>tpi</i>	A	8	362	12	1.33	1.28	0.21	87.6	11	5.22	4.84	0.39	0.017	0.009	0.096	0.085

Shown are the chromosomal location of the genes (dot, Y-linked, X-linked, or autosomal), the number of alleles sequenced (N), Polymorphism measured by π and θ , and $\times 10^{-2}$, length in base pairs (L), segregating sites (S), Tajima's D (D). Divergence (K_A , K_S , and K_A/K_S) is to *D. putrida*.

Table S6. HKA test results using MLHKA.

Species	Loci included	Selection on	k ($kl-5$)	LnL	df	LR	P -value	
<i>D. neotestacea</i>	<i>kl-5</i> , X, A, dot, Y	none	1.00	-84.09				
		<i>kl5</i>	0.04	-76.09	1	16	0.000	
		<i>kl-5</i> , dot, Y	0.08	-55.99	7	56.2	0.000	
	<i>kl-5</i> , X, A	none	1.00	-45.12				
		<i>kl-5</i>	0.01	-29.70	1	30.83	0.000	
	<i>kl-5</i> , Y, dot	none	1.00	-24.09				
		<i>kl-5</i>	0.19	-26.09	1	4.0	0.046	
	<i>kl-5</i> , dot	none	1.00	-16.14				
		<i>kl-5</i>	0.29	-17.76	1	3.2	0.073	
		<i>kl-5</i> , Y	none	1.00	-11.64			
	<i>D. orientacea</i>	<i>kl-5</i> , X, A, dot, Y	none	1.00	-79.58			
			<i>kl5</i>	0.17	-74.91	1	9.34	0.002
<i>kl-5</i> , dot, Y			0.15	-62.88	7	33.4	0.000	
<i>kl-5</i> , X, A		none	1.00	-41.01				
		<i>kl-5</i>	0.04	-32.65	1	16.72	0.000	
<i>kl-5</i> , Y, dot		none	1.00	-26.79				
		<i>kl-5</i>	1.42	-26.63	1	0.32	0.571	
<i>kl-5</i> , dot		none	1.00	-19.83				
		<i>kl-5</i>	1.35	-19.69	1	0.28	0.592	
		<i>kl-5</i> , Y	none	1.00	-12.13			
<i>D. testacea</i>	<i>kl-5</i> , X, A, dot, Y	none	1.00	-76.34				
		<i>kl5</i>	0.26	-74.34	1	4.00	0.0455	
		<i>kl-5</i> , dot, Y	0.23	-53.61	7	44.46	0.000	
	<i>kl-5</i> , X, A	none	1.00	-36.84				
		<i>kl-5</i>	0.07	-28.61	1	16.48	0.000	
	<i>kl-5</i> , Y, dot	none	1.00	-27.30				
		<i>kl-5</i>	1.89	-26.75	1	1.1	0.294	
	<i>kl-5</i> , dot	none	1.00	-21.41				
		<i>kl-5</i>	1.74	-20.96	1	0.9	0.34	
<i>kl-5</i> , Y		none	1.00	-11.32				
		<i>kl-5</i>	4.25	-10.66	1	1.32	0.251	

Outgroup was *D. putrida* for all comparisons. k is the selection parameter, and indicates the extent to which *kl-5* diversity is elevated relative to the neutral expectation based on divergence. LR is the likelihood ratio statistic of the model with selection compared to the model that assumes neutrality (no selection). Loci are as follows: X chromosome (*mof*, *pgd*, *rpl*, *skpA*), autosomal (*tpi*), dot (*bt*, *eIF4G*, *plexA*, *gw*), and Y-linked (*kl-2*, *kl-3*).

Table S7. McDonald-Kreitman tests for *kl-5*.

	nonsyn poly	nonsyn fixed	syn poly	syn fixed	neutrality index	codons	mean N	Fisher's exact test <i>P</i> -value
Unpolarized								
<i>orientacea</i> vs. <i>putrida</i>	5	16	9	71	2.465	682	38	0.161
<i>testacea</i> vs. <i>putrida</i>	2	14	8	68	1.214	679	41	0.684
<i>neotestacea</i> vs. <i>putrida</i>	1	13	1	63	4.846	682	50	0.329
Polarized with <i>putrida</i> and <i>falleni</i> as outgroups								
<i>orientacea</i>	3	4	6	15	1.875	264	38	0.646
<i>testacea</i>	0	3	5	16	0	264	41	1.000
<i>neotestacea</i>	1	3	1	15	5.000	264	50	0.368
Polarized with <i>putrida</i> and <i>phalerata</i> as outgroups								
<i>orientacea</i>	2	4	9	13	0.722	264	50	1.000
<i>testacea</i>	0	2	6	13	0	264	41	1.000
<i>neotestacea</i>	1	3	1	11	3.667	264	50	0.450

Figure S2. *kl-5* phylogeny from testacea group. Neighbor-joining tree with bootstrap values based on 1000 bootstrap replicates. Only bootstrap values >70% shown. A total of 2048 characters were used, of which 142 were variable and 129 were parsimony informative. Each sample is labeled by M or F to indicate male or female, the sample number, and then A1, A2, or Y to indicate autosomal allele number or Y-linked.

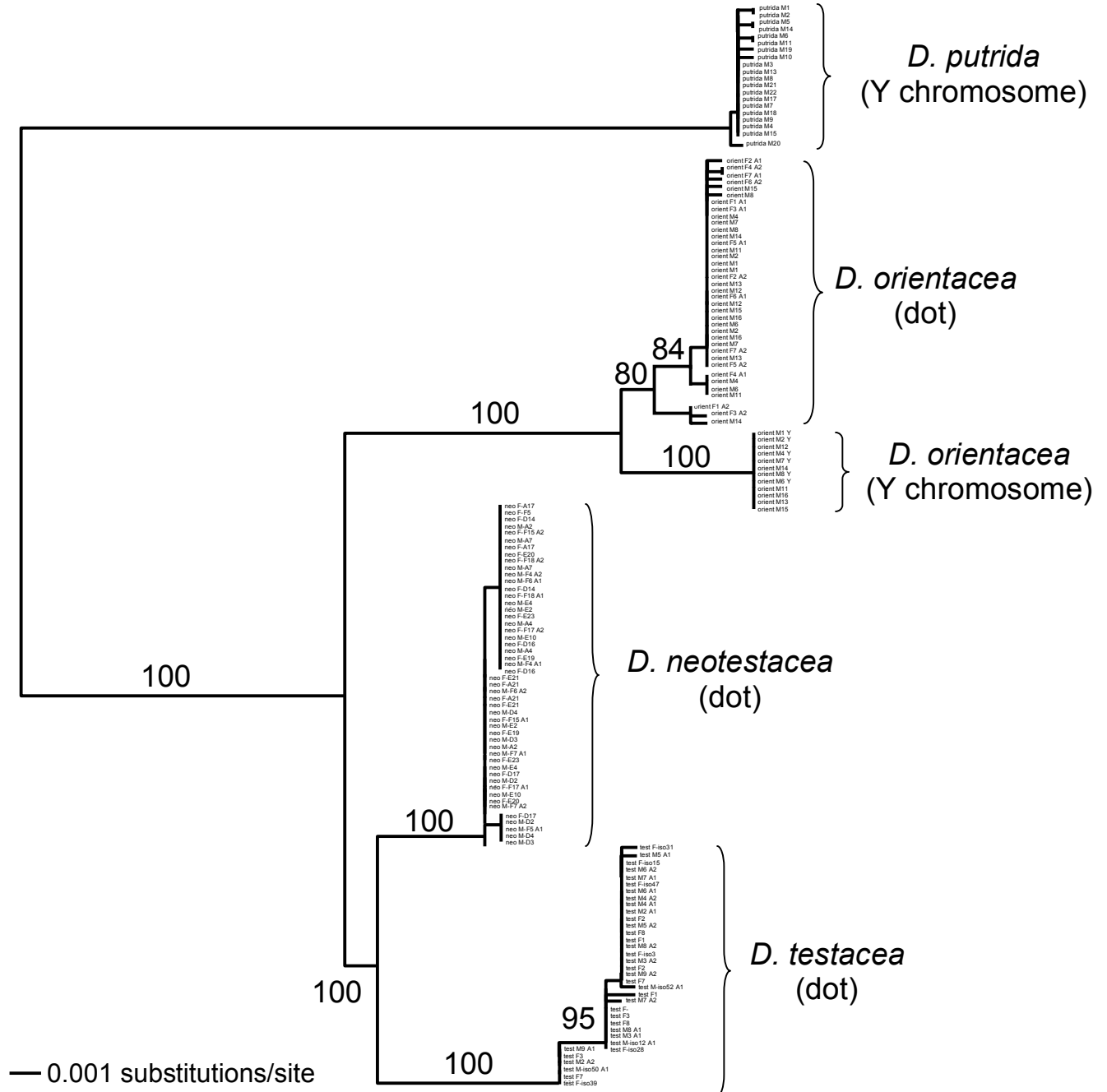
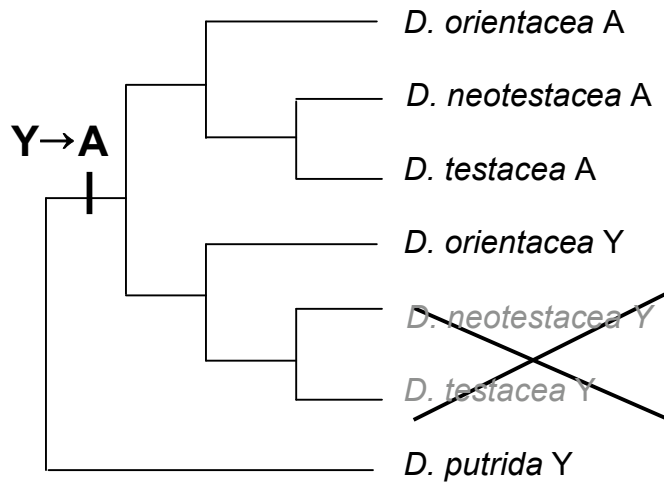
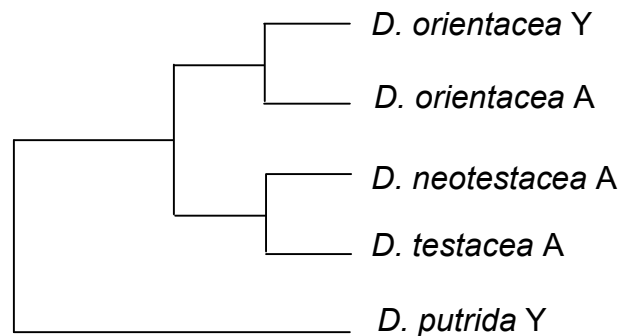


Figure S3.

(A) Expected scenario if *kl-5* moved once at the base of the three testacea group species and no further gene exchange occurred. The A and the Y after the species name indicate the genomic location, as autosomal (A) or Y-linked (Y). Note that because the *neotestacea* and *testacea* Y-linked copies of *kl-5* are no longer present, in this scenario we expect the Y-linked copy of *D. orientacea kl-5* to be basal to all the autosomal copies.

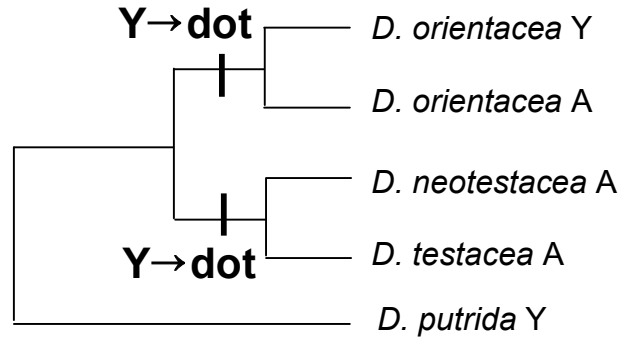


(B) Schematic of the observed *kl-5* phylogeny (see also Supplemental Figure S2 for the actual phylogeny of *kl-5* in the testacea group). Contrary to the above expectation, the Y-linked copy of *kl-5* in *D. orientacea* is very closely related to the autosomal copy of *kl-5* in *D. orientacea*, and is not basal to all of the autosomal copies.



(Figure S3 Continued)

(C) Evolutionary scenario #1: *kl-5* translocated off the Y chromosome twice and on to the dot chromosome: at the base of the testacea species and during the evolution of the *D. orientacea* lineage.



(D) Evolutionary scenario #2: *kl-5* moved from the Y to the dot in the common ancestor of the three testacea group species, and then from the dot to the Y during the *D. orientacea* lineage, either by a separate translocation or through gene exchange such as gene conversion or mitotic recombination.

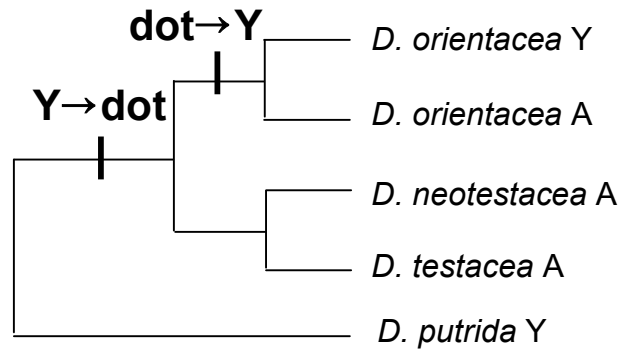
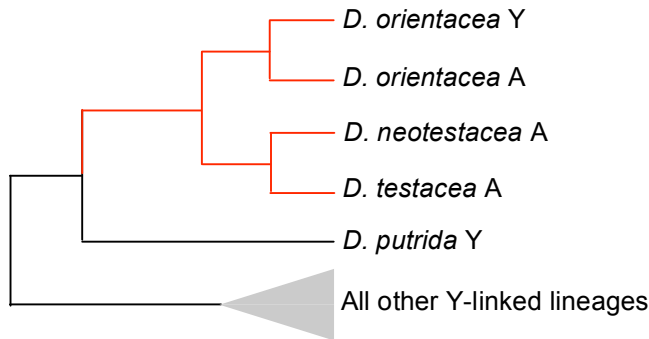
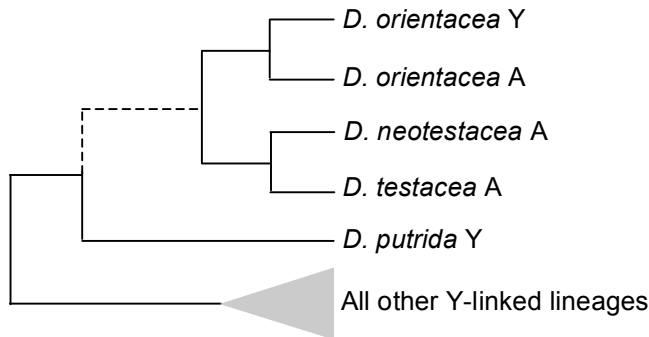


Figure S4. Visual representations of the evolutionary models used in the PAML analyses to test rates of evolution along different lineages of the *kl-5* phylogeny. The A and the Y after the species name indicate the genomic location, as autosomal (A) or Y-linked (Y). Model 1 has one ω along entire phylogeny. The other models include:

- (A) Model 2 has two ω values: one ω for the non-testacea group species plus the lineage to *D. putrida* (ω_1 in black) and a second for the testacea group (ω_2 in red), excluding the lineage to *D. putrida*



- (B) Model 3 has two ω values: one ω for the lineage leading to the common ancestor of *D. neotestacea*, *D. orientacea*, and *D. testacea* (ω_2 as dashed line), and another for the remaining lineages (ω_1 in black).



- (C) Model 4 has three ω values: one for the non-testacea group species (ω_1 in black), a second for *D. neotestacea*, *D. orientacea*, and *D. testacea* (ω_2 in red), and a third for the common ancestor of these three species (ω_3 as dashed line).

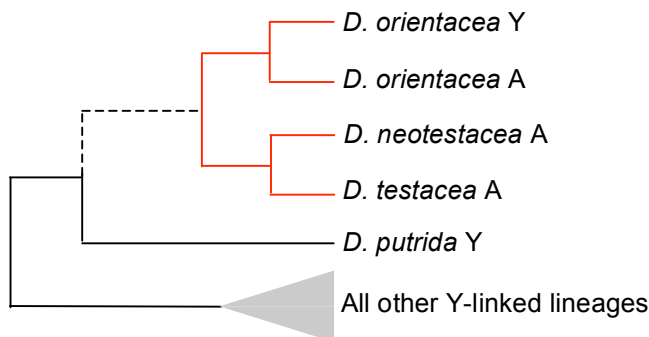


Figure S5. Comparison of α estimates. Boxes encompass the middle 50% of bootstrap values, with whiskers extending to the 95% confidence intervals. Values for Y-linked and dot loci and the dot-linked copy of *kl-5* are shown separately.

